

Foetal kidney vascular development of the mammal *Sus scrofa* *

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All vertebrates have a kidney and throughout the evolution of vertebrates the function of the kidneys has consistently been for the removal of nitrogenous waste and the maintenance of osmotic balance within the animal. However, mammals have specialised the kidney to allow for the concentration of urine above that of blood thus allowing for increased conservation of water. The functioning of the kidney relies on the correct structure of the nephron within the kidney. The basic vertebrate nephron contains a glomerulus encompassed by the Bowman's capsule, which passes the ultrafiltrate from the blood into the nephron, and the proximal tubule that reabsorbs molecules like glucose and other chemicals to be conserved by the body whilst actively secreting larger molecules such as penicillin. Finally the filtrate passes into the collecting duct which directs the final product for transport out of the ureter. As noted the mammalian kidney is able to concentrate urine through the reabsorption of water which occurs in the loop of Henle. The presence of a loop of Henle in a kidney indicates the capacity for a kidney to concentrate urine if needed.

Apart from the presence of the loop of Henle there is another difference in mammalian kidney structure/function and that is in the development of the kidney. Unlike other vertebrates, in mammals the developing kidney does not necessarily have to be fully functional during gestation due to the functioning of the placenta. Like other organs within the mammal (lungs and liver) it is possible then to delay development of the kidneys until close to birthing when they will need to be fully functional. The development of the vascularisation of the kidney and by association the kidney itself is something that has been discussed in a number of publications but has yet to be characterised visually. The aim of this study was to examine the kidneys of foetal pigs at different stages to characterise the rate and development of the glomeruli within the kidney and thus the rate and development of the nephrons and the overall structure of the kidney.

Foetal pigs were collected from the local abattoir and frozen before use. These pigs were separated into two age groups based on gestational size and included 68 days into gestation (9–10 weeks) and 114 days into gestation (16–17 weeks). A mid-ventral incision was made to gain access to the dorsal aorta just anterior to the kidneys. Gastric and mesenteric arteries were clamped off and a cannula inserted and tied into the dorsal aorta. 10% saline solution containing 1000 I.U. Heparin per

millilitre was flushed through the renal arteries via the cannula until the colouration of the kidneys change to pale indicating the removal of the blood. Mercox solution was mixed as per instructions and was injected into the renal arteries via the dorsal aorta until a colour change was noted based on the colour of the Mercox. The animal was then left to sit for an hour until the Mercox had hardened into a resin cast. For a period of a week the animal was placed in a bath of sodium hydroxide to allow all excess tissue to be digested leaving a cast of the kidney vascular system. The casts were cleaned in increasing concentrations of ethanol for storage before imaging took place. Macroscopic images were obtained using Olympus S2-CTV dissection microscope before smaller samples were extracted from the casts. Samples were dried using a Polaron critical point drier before being gold coated for electron microscopy. Images were taken using a Philips XL30 Scanning Electron Microscope at 5–10kv acceleration with a spot size of 3.

From the macroscopic images that were collected it was clear that there was a difference between the overall macro-anatomy of the late-stage kidney compared to the early-stage kidney. Unlike the late-stage kidney, with its clear cortex medulla differentiation, no such differentiation was shown in the early-stage kidneys. In some cases the glomeruli ruptured under the pressure of either the saline solution or the Mercox and a cast of the entire Bowman's capsule and nephron was inadvertently taken. From these extra casts it was shown that, although the nephrons are present in the early-stage kidneys the loops of Henle have yet to be developed. Upon closer examination of the glomeruli within the casts there are two noticeable differences between the late-stage and early-stage kidneys. First, there was a large difference in the average size of the glomeruli: the glomeruli in early-stage kidneys were found to be up to four times larger than the glomeruli found in late-stage kidneys. Second, the overall distribution of glomeruli also appears to have changed: once the cortex/medulla differentiation was taken into account it was also seen that the number of glomeruli based on density has also increased in the late-stage kidneys.

Without a clear cortex/medulla differentiation in the early-stage kidney it is clear that the early-stage kidney does not have the capacity to concentrate urine as a typical adult mammalian kidney would. A cast of a nephron in the early-stage kidney was also captured and showed no loop of Henle which further indicates no capacity to concentrate urine. The cast of the early-stage nephron however does indicate the possibility of a functional kidney even at this early stage of development. The functioning of the available glomeruli relies primarily on the overall surface area of the glomeruli as a whole and as such it was unexpected to have such a dramatic decrease in the size of individual

* Extended abstract of a paper presented at the Royal Society of Western Australia Postgraduate Symposium 2013 held at Murdoch University on 12 October 2013.

glomeruli. A decrease in size may increase overall pressure and filtration capacity of the glomeruli and an increase in complexity and glomeruli density ultimately increases surface area of the glomeruli in the entire kidney. The overall structure of the late-stage kidney was as expected when compared to the known structure of the adult mammalian kidney. This structure was expected as the late-stage foetal pigs were near birth and would need a fully functional kidney for survival. Within the earlier stage kidneys however we have seen what is essentially a structurally sound (and possibly functioning kidney) but one that is significantly different to that of an adult mammal. Comparisons with the structure of adult non-mammalian (reptilian) kidneys, however, show a

structure that is similar in many aspects to the early-stage kidney of the foetal pig which may be a case of ontogeny recapitulating phylogeny.

ACKNOWLEDGEMENTS

We acknowledge the facilities, and the scientific and technical assistance of the Australian Microscopy & Microanalysis Research Facility at the Centre for Microscopy, Characterisation & Analysis, University of Western Australia, a facility funded by the University, State and Commonwealth Governments. We would also like to acknowledge the help and advice of Tom Stewart.